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I. Wissenschaftliche Mittheilungen.

1. The Life-History of Dicyema[1].

By William Morton Wheeler.

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Our knowledge of the singular parasites known as *Dicyemidae* is very largely embodied in two admirable monographs of the European species, one by Prof. Ed. van Beneden (1876), the other by Prof. C. O. Whitman (1883). With the exception of a very brief note on the forms which I found in *Octopus punctatus* of the Californian coast (1897), no observations have as yet been published on the American species. Although my studies are still incomplete, I have decided, nevertheless, to present some of my conclusions, since on at least one point relating to the reproduction of *Dicyema* they lead to a new conception of the animal's life history.

The very simple structure of *Dicyema* may be briefly considered before passing to the question of its mode of reproduction. The body of the animal is long and worm-like and consists of two portions, one of which is a single long, narrow cell, tapering at its ends, the axial, or entoderm cell, the other a single layer of cells completely investing the axial cell and known as the ectoderm. These names, given in the

[1] A preliminary paper read at the annual meeting of the American Morphological Society, Dec. 30th, 1898.

days of germ-layer cult, suggest theoretical conceptions which may be inapplicable to the *Dicyemidae.*

In the ectoderm five zones, each consisting of several cells, follow one another in regular sequence from the anterior to the posterior end. The odd-numbered zones, i. e. the first, third and fifth are constant in the number of their cells in all known *Dicyemidae.* Of the even-numbered zones, the second exhibits a generic, the fourth a specific variation in the number of its constituent elements. Thus in all known *Dicyemidae* in the first zone there are four cells known as the propolars. The second, or metapolar zone may consist of four five, or six cells. The pro- and metapolar zones taken together form the "calotte", which may, therefore, be octamerous (in *Dicyema*), enneamerous (in Prof. Whitman's genus *Dicyemennea*) or decamerous (in a new genus which I shall call *Dicyemodeca*). The three genera are represented each by a single species in the *Octopus* of the Pacific coast (*Dicyema coluber* n. sp., *Dicyemennea Whitmanii* n. sp., and *Dicyemodeca sceptrum* n. sp.). The calotte of the European Dicyemids may be either radially (orthotropal) or bilaterally symmetrical (plagiotropal). All the American species known to me have orthotropal calottes. The third zone always consists of two cells, the parapolars. The same is true of the fifth zone, or uropolars as they may be called. Between the parapolars and uropolars extends the longest and most variable region, consisting of from ten to eighteen cells in different species. These cells, which I shall call diapolars envelop the axial cell as a spiral band, a single revolution of which may consist of either two or three of these cells according to the species. As the animal grows older certain diapolars, the uropolars and in some cases also the parapolars may become filled with refractive granules and bulge out beyond the remaining ectoderm cells. In this condition they are called verruciform cells. Their function is unknown.

The calotte, which is more densely ciliated than the remaining ectoderm, attaches the Dicyemid to the surface of the venous appendages of its Cephalopod host, while the body of the parasite floats out in the renal liquid. When the parasites are abundant they form a pilose or velvety coating to the venous appendages. When detached they move about in the renal liquid, bending their protoplasmic bodies easily and gracefully. *Dicyema coluber*, which attains a length of 2—5 mm, is able to coil itself up like a watch-spring.

The reproductive elements make their appearance and develop within the axial cell, all known *Dicyemidae* being viviparous. Besides the large nucleus the axial cell of the fully formed Dicyemid, even

before it leaves its mother, always contains germ-cells or developing embryos. A study of these has led to the distinction of two very different lines of embryonic development in individuals of the same species. In some of the individuals the germ-cells develop into so-called vermiform, in others into what are called infusoriform embryos. Although it has been shown (Whitman) that the parents of the two kinds of embryo are indistinguishable morphologically, it has, nevertheless, been found convenient to give them different names. The mother of vermiform embryos is colled a nematogen, the mother of infusoriform embryos a rhombogen.

The vermiform embryos arise in the entoderm cell of the nematogen in the following manner: In the earliest stages a single germ-cell exists in the protoplasm of the axial cell. This germ-cell divides, its daughter cells separate and divide in turn, and the process continues till the axial cell contains a number of isolated germ-cells. Then, from some unknown cause, this method of division with subsequent separation of the cells changes abruptly. Each germ-cell divides but the daughter cells henceforth remain together as blastomeres of a regularly segmenting ovum. The cell-mass thus produced has been interpreted as an epibolic gastrula with a single central cell as its entoderm. The spherical or elliptical cell-mass elongates after the specific adult number of cells is formed and we have the vermiform embryo, which is a miniature of its mother. When mature it passes out through the ectoderm cells of its parent into the renal cavity of the Cephalopod. Sometimes the embryo does not leave its mother till it contains within its own axial cell another vermiform embryo (van Beneden), so that we may have a true case of emboîtement like that seen in *Gyrodactylus* among the Trematodes.

The offspring of the rhombogenic mother, viz. the infusoriform embryos, are produced in a different manner, although they too start from germ-cells like those to which the vermiform young can be traced. In the rhombogen however, the germ-cells are few in number and lie at intervals in the axial cell. Each of them begins to proliferate by a regular cleavage and passes through an epibolic gastrula to a stage resembling the very young nematogen and consisting of a single large entoderm cell enveloped by a layer of ectoderm cells. At this stage a sudden change occurs in the development, the very opposite of that which supervenes in the development of the vermiform embryo. The ectoderm cells free themselves gradually one by one and pass off into the protoplasm of the axial cell in such a way that they form a series on either side in the direction of the long axis of the mother. While they are passing off as rounded isolated cells, the cell-mass

from which they come known as the infusorigen, steadily increases in size through division of the elements surrounding the central cell. The cells that have freed themselves from the infusorigen undergo a regular cleavage which results in a second epibolic gastrula. This develops into the infusoriform embryo. As the series on either side continually receives new additions at its starting point, we have radiating from the infusorigen two rows of embryos which become older and older the greater the distance from their common source.

The fully developed infusoriform has a remarkable structure first elucidated by van Beneden (1876). It is a distinctly bilateral organism nearly as broad as long, rounded anteriorly and somewhat pointed posteriorly. It consists of two portions, what van Beneden has called the "urn", which corresponds in position with the entoderm cell of the vermiform embryo, and a covering of ectoderm cells. The ectoderm cells are curiously differentiated. Those occupying the anterior third of the body are smooth and eciliate, the others ("corps ciliaire" of van Beneden) are provided with prowerfull cilia. Mereover two of the anterior smooth cells are greatly enlarged and each contains a huge refractive body of unknown function. The urn, too, has a rather complicated structure. It consists of a capsule with a lid and contents. This capsule, which is to the contents what the skin of an orangs is to the pulp, consists of two concave cells. The lid which closes the spherical capsule, is composed of four flattened cells, each the quadrant of a circle. The nuclei of these cells are said to disappear by van Beneden, who also describes certain peculiar details in their structure which I have not been able to recognize as constant features in *Dicyema coluber*. The contents of the urn consists of a body originally composed of four cells, each the quarter of a sphere, but the four nuclei of these cells soon multiply and steadily decrease in size till the urn is filled with a mass of minute, very deeply staining granules.

The foregoing is the substance of our knowledge of the structure and life history of the *Dicyemidae*. In again taking up the study of these neglected parasites I have attempted to find answers to three questions:

1) Are the nematogenic and rhombogenic individuals two distinct kinds of female, or are they only different phases of the same individual Dicyemid?

2) What is the meaning of the "infusoriform embryo"?

3) What is the meaning of the "infusorigen"?

1) Van Beneden answers the first of these questions to the effect that there are two forms of female, comparable to the dimorphic females of the *Orthonectidae*. This is hardly more than an attempt

to explain the obscure by the more obscure. Prof. Whitman, too, maintains that there are two kinds of females, but not in the sense of van Beneden. He calls certain females monogenic, or primary nematogens, on the supposition that they produce only vermiform young. Others, he believes, are first rhombogens and later in life nematogens. These he calls diphygenic.

My own study of *Dicyema coluber* leads me to adopt a very different and a much simpler view. I began by preserving in formalin all the *Dicyemidae* taken from a hundred *Octopus punctatus* of different sizes, and by noting the character of the embryos in each case. The results of this work are given greatly condensed in the following table[2]:

56 *Octopus* 1—3 cm long. Contained only nematogens, the number of the parasites increasing with the age of the host.

6 *Octopus* 3,5 cm long. Nearly all nematogens, a few young rhombogens (i. e. with young infusorigens).

8 *Octopus* 4—5 cm long. Nematogens and rhombogens, the latter often preponderating, but always young.

6 *Octopus* 5,5—6 cm long. Mostly rhombogens with more advanced infusoriform embryos; very few nematogens.

21 *Octopus* 7—12 cm long. Rhombogens except in very rare cases when a few nematogens were found; no nematogens in the larger specimens of *Octopus* (10—12 cm).

3 *Octopus* 22—25 cm. All rhombogens with very old infusoriform embryos and old infusorigens; axial cell often empty for long distances as if exhausted of its embryos.

From these observations I inferred, first, that the same *Dicyema* is both nematogen and rhombogen at different periods of its life, and second, that it is first a nematogen and then a rhombogen, i. e. it develops in the reverse order of Prof. Whitman's sequence.

I next confined my attention to the *Dicyemidae* taken from Cephalopods 4—5 cm long, the stage in which the transformation of the nematogen into the rhombogen should occur if my inference was correct. In searching through a great amount of material I succeeded in finding some ten or twelve *Dicyema* which certainly

[2] The measurements of the Cephalopods were taken from the posterior end of the body to the middle of a line joining the anterior edges of the eyes, so that the length of the arms is not included. The method of weighing adopted by Prof. Whitman would have been preferable to measuring these contractile mollusks, but no satisfactory balances were at hand when I was working at San Diego, Cala, where most of my material was collected.

contained both vermiform and infusoriform embryos in process of development. This, of course disposes of van Beneden's view of dimorphic females, and indicates a transition between the two reproductive phases.

That the sequence is from the nematogenic to the rhombogenic phase and not vicê versâ is also indicated by the detailed study of the contents of the axial cell in the transitional stages. Only a portion of the germ-cells gives rise to vermiform young in the nematogenic mother, so a stage is reached in which the axial cell is filled with vermiform embryos plus a number of isolated germ-cells. This stage is especially common in *Octopus* 4—5 cm long. I believe that many of these germ-cells then leave the parent, for somewhat later one finds the diapolar ectoderm cells each containing as many as four or five nuclei. These resemble germ-cell nuclei and can only come from the axial cell since the ectoderm nuclei are never seen dividing even by amitosis. As these nuclei finally disappear they must be either expelled into the renal liquid or absorbed by the protoplasm of the diapolar cells. The germ-cells in the protoplasm of the axial cell, thus greetly reduced in number, come to be separated from one another by long intervals. They then undergo cleavage to form the infusorigens, of which there may be as many as five or six in one *Dicyema*, and these in turn produce the infusoriform young. As a rule all the vermiform embryos have left the parent before the infusorigens appear, but as in the ten or twelve cases above mentioned, the development of the two kinds of offspring may overlap, unmistakable infusoriform embryos coexisting with vermiform embryos in the axial cell of the same *Dicyema*.

2) As Balfour (1881) has said, the taxonomic position of the *Dicyemidae* must depend on the interpretation of the infusoriform embryo. Properly speaking this is not an embryo at all, but must be either a larval or adult form since it escapes from its mother and is able to move about for some time in the renal secretion or in the sea-water. I agree with van Beneden (1882) in interpreting this singular organism as the male Dicyemid. That there are strong grounds for this view is evident when we compare the infusoriform with what is certainly the male of the *Orthonectidae*, the nearest allies of the *Dicyemidae*. The male Orthonectid (*Rhopalura*) combines characters of the vermiform, i. e. of the female, with those of the infusoriform Dicyemid. It is long, fusiform and radially symmetrical like the vermiform, but, like the infusoriform, contains a central body full off granules. Its ectoderm, moreover, is composed partly of smooth, eciliate cells, each containing a refractive body, and partly of powerfully ciliated

cells. In both there are certain problematic cells between the granular body and the ciliated ectoderm. That the infusoriform should be bilaterally instead of radially symmetrical, and have only two huge cells containing large refractive bodies instead of many small ones with small refractive bodies is no serious obstacle to the comparison. The granules in the central body of *Rhopalura* are true flagellate spermatozoa as Metchnikoff (1881) has shown, although Julin (1882) was less successful in resolving them. I have had no better success than van Beneden in making out flagella to the granules in the urn-contents of the infusoriform. Van Beneden, however, believes that he has seen a ciliary movement in the urn. The contents of this mysterious organ are discharged by the infusoriform after it leaves its mother. In the Californian species the infusoriform rarely remains within its mother till the small nuclei of the urn-contents are reduced to granules, so I am inclined to believe that the spermatozoa, if spermatozoa they really be, must mature while the infusoriform is swimming about.

3) The signification of the infusorigen is even more obscure than that of the infusoriform. It has been regarded as an embryo (epibolic gastrula) the ectoderm cells of which are all germ-cells and give rise to the infusoriforms. On this supposition *Dicyema* performs on its offspring an experiment which some experimental embryologists would regard as impossible, since in this case the gastrula is resolved into its component cells each of which is capable of reproducing a whole organism. I believe that the infusorigen may admit of a different interpretation. I find both in sections and in *Dicyema* mounted in toto, a number of very deeply staining granules mingled with and adhering to the cells of the infusorigens. These granules closely resemble the minute bodies contained in the urn of the mature infusoriform and may therefore be spermatozoa. If this is the case, the central cell of the infusorigen may represent a chemotactic center which facilitates fertilization by holding all the germ-cells together in a mass and by attracting the spermatozoa to the same point in the axial cell of the *Dicyema*. On this interpretation the male Dicyemids would arise from fertilized, the females, or nematogens from unfertilized ova.

In conclusion I will give the life-history of *Dicyema* as suggested by the preceding considerations. Both the female (nematogen) and the male (infusoriform) may migrate from one *Octopus* to another. One or more nematogens probably enter the kidney soon after the *Octopus* is hatched and these multiply paedogenetically till the surfaces of the venous appendages are tufted with nematogens. After a time parthenogenesis languishes, the germ-cells being no longer able to produce

vermiform embryos. By this time one or more males from some other *Octopus* find their way into the kidney and discharge their spermatozoa. These spermatozoa enter the axial cells of the nematogens and fertilize the germ-cells aggregated in the infusorigens. From these cells develop the males which may in turn fertilize either the remaining nematogens in the same kidney or migrate to other Cephalopods. As in so many other cases in the animal and vegetable kingdoms the males make their appearance when the conditions of life become unfavorable, viz. after the kidney is well peopled with Dicyemids and food is less abundant. These unfavorable conditions are perhaps still further aggravated by changes in the renal metabolism of the *Octopus* when it is about 4 cm long. This view of the lifehistory of *Dicyema* dispenses with the postulate of an intermediate host and an adult form of which *Dicyema* is only the larva. The *Octopus* of the Pacific coast, at least in its youth, is more or less gregarious. I have taken more than fifty small individuals (1—5 cm long) in an area of less than an acre in San Diego Bay. Under such conditions even minute parasites like *Dicyema* would, perhaps, find little difficulty in migrating from one *Octopus* to another.

While it simplifies our views of the life history of the Dicyemids, the above interpretation complicates the question of their systematic affinities. Their mode of reproduction would appear to be very unlike that of the flat-worms from which many zoologists derive them. In my opinion, it is a step backwards to place the *Dicyemidae* in a separate subkingdom, as did van Beneden when he called them Mesozoa. I believe, however, that their structural and developmental peculiarities entitle them to a more independent rank than that of an appendix to the Platyhelminthes.

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